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Haemotological Studies on Aquacultured Shrimp Species from Tamilnadu, India

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Abstract

In general, the Total Haemocyte Count (THC) and Differential Counts (DC) could be used to understand the health status of shrimps. The present study was undertaken with the prime objective of investigating the variations of Total Haemocyte Counts (THC) and Differential Counts (DC) in both apparently healthy and diseased *Fenneropenaeus indicus* and *Penaeus monodon* from farms along the Tamil Nadu coast. In the hematological studies, three types of haemocytes such as Hyaline Cells (HC), Small Granulocytes (SG) and Large Granulocytes (LG) were observed. In *Fenneropenaeus indicus* and *P. monodon,* Small granulocytes, large granulocytes and Hyaline Cells contributed about 15%, 65% and 20% of Total Haemucyte Count respectively. In apparently healthy *F. indicus and P. monodon,* the mean values of THC was recorded to the tune of 8251.56 ± 936.9 and 8147.75 ± 860.01 cells / mm^3 respectively. In infected *F. indicus and P. monodon,* the mean values of THC was recorded to the tune of 2247 ± 426.41 and 2763 ± 280.31 cells / mm^3 respectively. The THC of diseased shrimps were found to be lower than the apparently healthy shrimp. In DC studies, the mean of hyaline cells in disease affected *F. indicus* (7.00 %) and in *P. monodon* (7.22%) in affected shrimp markedly increased to 18.75% and 18.89% respectively than apparently healthy shrimps. In *F. indicus* and *P. monodon,* the mean of small granulocytes showed a decrease trend of 54.50% and 54.22% in affected shrimps when compared to the apparently healthy shrimps (71.00 % and 72.88%) respectively. The mean of large granulocytes increased from 20.37% to 25.50% and 19.88% to 23.550o in normal and affected *F. indicus* and *P. monodon* respectively. Students 't' test affirmed that between normal and infected *F. indicus,* THC ($P<0.0$ I), HC ($P<0.01$), SG ($P<0.01$) and LG ($P<0.02$) had significant difference. Similarly, between normal and infected *P. monodon,* significant difference was observed in THC, HC, SG and LG ($P<0.01$).

Key words : Haemolymph, THC, Large granulocytes, Small granulocytes - *F. indicus* and *P. monodon*

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Introduction

Shrimp culture is one the most important beneficial trade sector in India. The intensive culture systems which adopted by shrimp farmers frequently cause stress to the shrimps and consequently, predispose them to infection (Kakoolaki *et al.*, 2010). Shrimp health is influenced by a range of factors, and one of the most important being the environmental stress. Wide range of' environmental factors such as water quality parameters and mismanagement of the ecosystem leading to stress are the root cause of most shrimp diseases. More than 20 viral species have been identified from diseased shrimps (Lightner, D. V., 1992). In culture systems, health problems such as reduced growth, abnormal behavior and widespread mortalities are usually detected at an advanced stage as reported by Perazzolo *et al.*, (2002). It is evident that certain diseases and toxins cause changes in blood characteristics as in vertebrates. The haemolymph is the accessible internal tissue in shrimp it contains different types of cells such as hyaline or a granular, small granular and large granular haemocytes (Martin and Graves, 1985). These cells play vital role in wound repair (Fontaine and Lightener, 1973) and clotting of haemolymph (Wood *et al.*, 1971). Shrimp lack specific acquired immunity, the nonspecific activity mediated by the circulating blood cells appears to be of considerable importance in resistance to disease (Sinderman, 1971). Disease prevention has been a priority in shrimp farming and shrimp immunology has become a prime area of research. In shrimp, the most important role of the circulating haemocytes is the protection of animals against invading microorganisms by participating in recognition, phagocytosis and melanization (Tzou *et al.*, 2002; Cerenius and Soderhall, 2004; Hsieh *et al.*, 2008). Shrimp hemocytes are involved in defense mechanisms such as phagocytosis, encapsulation, clot formation and melanization (Johanson *et al.*, 2000). Thus hemocytes counts are used as an indicator of shrimp health status (Perazzolo *et al.*, 2002). Jago *et al.*, (2004) also advocated that THC and DC can be used in assessing the stress or health status of lobsters. The present study was undertaken with the prime objective of investigating the Total Haemocyte Counts (THC) and Differential Counts (DC) in normal and diseased *F. indicus* and *P. monodon* and to find the response of the haemocytes with respect to infection. The aim of this study was to understand the health status of cultured shrimp and to develop a simple and rapid method to estimate the health status of shrimps through the variation in cell counts and apply the methodology at field level to understand the physiology of shrimp in farm level and to design management procedures.

Material and Methods

A total of 80 apparently healthy and 80 diseased *Feneropenaeus indicus and P. monodon were* randomly collected from farms 16 of Tamil Nadu. The average size of shrimp is about 12±3.5 g. They were collected in oxygenated polythene bags and brought to the laboratory and maintained up to 48 hours with water salinity 32 ppt, temperature 25 to 28°C and oxygen level above 4mg/l with recirculatory facility and fed with commercial feed. From the conditioned shrimps the haemolymph was collected for Total haemocyte count (TUC) and Differential Count (DC) studies as per the technique described by Martin and Craves (1985). The diseases in the infected shrimps were confirmed by histopathological methods, The observed values are tested stastically.

Results and Discussion

The affected shrimps were found to be infected with Monodon Baculo Virus (MBV), White Spot Syndrome Virus (WSSV) Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) and the bacterial diseases vibriosis. The THC and DC in apparently healthy and diseased shrimp varied significantly. In the present study three types of hemocytes were identified through phase contrast microscopy in *F. indicus* and *P. monodon.* they are Agranular (hyaline), small-granular, and large-granular cells. Hyaline Cells or agranular cells (HC) represented about 10-15%, Small Granular Cells (SGC) comprised about 60 to 65% and the large granulocyctes forms about 15-20% of the total circulating hemocytes in *F. indicus and P. monodon.* Hyaline cells were found to be in round, ovoid, or fusiform in shape. They had dense cytoplasm and red in color with no granule in the cytoplasm. The high nucleocytoplasmic ratio of the HC was useful for its identification. Small granulocytes are ovoid or fusiform cells and they had low nucleuocytoplasmic ratio. Small granular haemocytes, had multiple small cytoplasm granules and the large granular cells had larger cytoplasmic granules. Horse *et al.,* (1990) classified the haemocytes based on morphological features in to hyaline haemocytes and large granulocytes. Martin *et al.,* (1985) described three types of haemocytes in Ridgeback prawn *Steyonia ingenitis.* Hose *et al.,* (1990) and Kondo *et al.,* (1998), classified the hemocytes of the shrimp (Penaeidae) into three types, Hyaline Cells (HC), Small Granular Cells (SGC) and Large Granular Cells (LGC) based on the cytoplasmic deposit and size of granules. He also observed that Hyaline cells, which are the smallest cells in *F. indicus* are easily lysed and they were in round or oval shape with a high nucleocytoplasmic ratio.

The information's collected from disease affected shrimp farms are furnished in Table 1. The comparisons of THC between apparently healthy and infected *F. indicus* and *P. mondon* are depicted in Fig. 1 and 2 respectively. As per the student's t-test analysis, the mean values of Total Haemocyte Counts (THC) in apparently healthy *F. indicus* and *P. mondon* examined are furnished in Table 2 and 3. The mean values of Total Haemocyte Counts (THC) in apparently healthy *F. indicus* and *P. mondon* were 8706.55±936.09 and 8147.75±860.01 cells/mm^3 respectively. Similarly, the mean value of THC in infected *F. indicus* and *P. mondon* was 2247±426.41 and 2763±280.31 cells/mm^3 respectively. In the present study, there was a marked reduction in the number of circulating haemocytes in the affected *F. indicus* and *P. monodon* when compared to that of apparently healthy shrimps. Students t test affirmed that between normal and infected *F. indicus,* THC ($P<0.01$). HC ($P<0.01$), SG ($P<0.01$) and LG ($P<0.01$) had significant difference (Table 2). In the present study, the decrease in THC may be due to the decrease in small granulocytes which was corroboration with the observation made by Song *et al.,* (2002), who reported that the THC of TSV-infected shrimp was decreased by 79% relative to normal shrimp. Similar observation was also made by Moullac *et al.,* (2005) who reported significant decrease in THC due to the decrease in small granulocytes and the Hyaline cells in *P. stylirostries* in response to hypoxic stress. The THC of *P. monodon* and *P. japonicus* infected with WSSV also decreased significantly (Hennig *et al.,* 1998). In contrary to above observation Selvin *et al.,* (2004) reported increase in THC to about 9185.71 cells/mm^3 in comparison to normal shrimp 5437.71 cells/mm^3. This may be due to the phagocyte effect of the haemocytes as reported by Cornick and Stewart (1968). Newman and Feng

Table 1 : Observation recorded from the aquaculture farmas.

Farm No.	Location	Total water spread area(ha)	Type of farming	Water quality				Source of water sea/ brackish water	Species cultured	Weight of shrimp (g)	Stocking density/m²	Water colour	Age (in days)
				pH	DO (ppm)	Temp °C	Salinity (ppt)						
1.	Madras	5	Modified extensive	8.2	2.4* 5.5**	30	34	Brackish water	*P. indicus*	8-10	15	Transparent	45
2.	Madras	7	Modified extensive	8.4	2.40* 6.25**	30	30	Brackish water	*P. indicus*	7-12	20	Light Green	50
3.	Tuticorin	5	Semi-intensive	8.6	2.2* 5.6**	29	36	Sea	*P. indicus*	10-12	25	Light Green	50
4.	Tuticorin	12	Intensive	8.2	2.8* 6.0**	28	36	Sea	*P. monodon*	7-10	45	Light green	52
5.	Pondicherry	10	Extensive	8.0	2.6* 6.0**	30	36	Sea	*P. monodon*	12-15	5-7	Transparent	50
6.	Tiruchendur	4.5	Extensive	8.6	2.5* 5.4**	30	37	Sea	*P. monodon*	18-22	8	Light green	45
7.	Poompuhar	40	Modified	8.5	2.8* 6.0**	29	36	Sea	*P. monodon*	10-12	15-20	Greenish	50
8.	Poompuhar	23	Semi-intensive	8.2	2.2* 5.7**	30	36	Sea	*P. monodon*	12-14	20	Greenish	70
9.	Tiruchendur	21	Modified extensive	8.0	2.6* 5.5**	29	36	Sea	*P. indicus*	8-10	10	Greenish	45
10.	Ramnad	5	Modified extensive	8.4	2.8* 7.0**	28	35	Sea	*P. monodon*	26-25	15	Greenish	90
11.	Ramnad	20	Intensive	9.0	3.0* 7.5**	29	36	Sea	*P. monodon*	10-12	30	Greenish	75
12.	Vedaranyam	15	Semi-intensive	7.0	2.0* 4.8**	29	34	Brackish water	*P. monodon*	11-15	25	Yellowish brown	75
13.	Pattukottai	18	Modified extensive	8.5	3.0* 6.5**	28	15	Brackish water	*P. monodon*	30-35	12	Yellowish green	110
14.	Tiruchendur	7	Modified extensive	7.8	2.8* 5.8**	28	32	Sea	*P. indicus*	8-10	9	Greenish	30
15.	Chennai	12	Semi-intensive	8.3	2.9* 6.0**	28	31	Brackish	P. indicus	8-10	12	Greenish	42
16.	Ramnad	18	Intensive	8.7	3.1* 7.5**	28	33	Sea	*P. indicus*	9-10	15	Yellowish green	47

*Note: *Morning; **Evening*

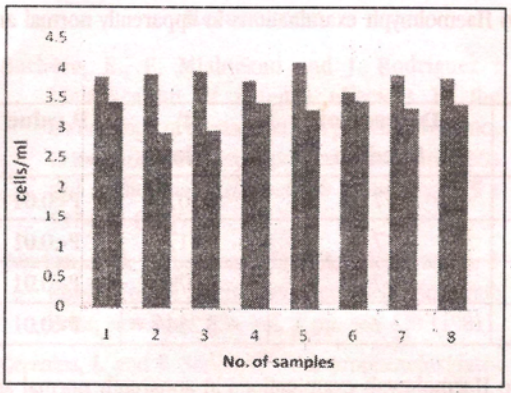

Fig. 1 : Comparation of THC between appratently healthy and infected *Feneropenaeus indicus*.

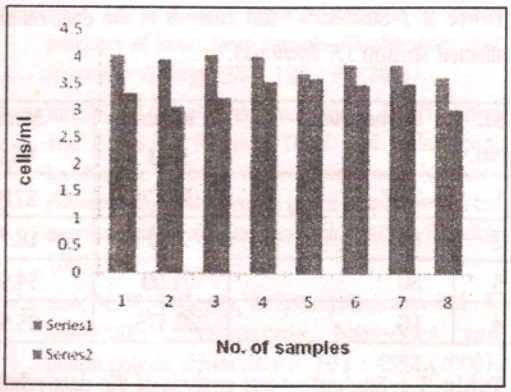

Fig. 2 : Comparation of THC between appratently healthy and infected *Penaeus monodon*.

(1982) reported marked decrease in total haemocyte count in different species of infected shrimps and also found considerable evidence that cellular defense mechanism plays main role in removal of foreign particles. Lightner *et al.*, (1983) reported that though there was an enhancement of cells due to haemotopoiesis subsequent to infection, but at later stages, there was an acute drop in circulating haemocytes.

The haemolymph of *F. indicus* and *P. monodon* comprise three major types of blood cells including hyaline cells, Small Granule Cells (SGC) and Large Granule Cells (LGC). The mean value of hyaline haemocytes in apparently healthy *F. indicus* and *P. monodon* (7.0% and 7.22%) markedly increase to about 18.75% and 18.89% in infected shrimps. Students t test affirmed that between normal and infected *P. monodon*, significant difference was observed in THC, HC, SG and LG ($P<0.01$). This observation was similar to that of Selvin *et al.*, (2004) who reported increase in hyaline cells to about 17.35% in infected shrimp than in control. Hyaline cells were considered to have phagocytic function (Soderhall and Cerenius, 1992). The increase in hyaline haemocytes in the affected shrimps could be due to simultaneous separation process triggered during infection as these cells were responsible for cuticular formation (Horse *et al.*, 1990).

The mean value of small granular haemocytes showed a decrease in affected shrimps (54.50±5.75) and (54.22±1.94) when compared to that of apparently healthy shrimps (71.00±1.36) and (72.88±1.36) in *F. indicus* and *P. monodon* respectively (Table 2 and 3). The marked reduction in the circulating small granular haemocytes in the affected shrimps might be due to higher virulence of the infective agents. Similar observation was made by Selvin *et al.*, (2004), who reported significant reduction of small haemocytes in infected shrimp to about 6.21 % from 15.57% in normal shrimp. Granulocytes were the main defense system against foreign particles because of their ability of phagocytosis (Hose and Martin, 1989). The semi granular haemocytes were reported to be the primary cells involved in phagocytosis of foreign particles of shrimp (Bachere *et al.*, 1995). However, it seems hyaline cells initiate some reaction such as clot formation. Lavine and Strand (2002), Cerenius & Söderhäll (2004) and Iwanaga and Lee (2005) proved that these reactions are often observed to

Table 2 : Student's t-test analysis of the data relating to Haemolmyph examinations in apparently normal and affected shrimp (*F. indicus*).

Sl. No.	Parameters	Mean (X_1)	Mean (X_2)	Degrees of freedom	't' value	P value
1	THC	8147.75	2719	7	5.5076	P<0.01
2	HY	7.00	18.75	7	6.0177	P<0.01
3	SG	71.00	54.50	7	7.3790	P<0.01
4	LG	20.375	25.50	7	3.2544	P<0.01

Table 3 : Student's t-test analysis of the data relating to Haemolmyph examinations in apparently normal and affected shrimp (*P. monodon*).

Sl. No.	Parameters	Mean (X_1)	Mean (X_2)	Degrees of freedom	't' value	P value
1	THC	8706.556	2763.000	6	6.1290	P<0.01
2	HY	7.222	18.8900	6	5.5513	P<0.01
3	SG	72.889	54.2222	6	7.5855	P<0.01
4	LG	19.889	23.5556	6	2.2454	P<0.01

become melanized, through the action of phenoloxidase.

In the present study, though there was moderate increase in large granular haemocytes which played important role in phagocytosis and encapsulation, the mean value of large granulocytes in the haemolymph of *F. indicus* and *P. monodon* in apparently healthy and diseased shrimp is 19.88±2.3 and is 20.37±2.5. Large granulocytes are also capable of phagocytosing foreign material but with less frequency than the smaller ones. Thus lesser variation in large granulocytes cells is observed which may be due to lesser involvement in defense or phagocyte activity. Jussila *et al.*, (1997) reported that the proposition of granulocytes declined to the lowest in moribund lobsters after they were held for 16 hrs in tanks in both healthy and diseased condition. Selvin (2005) reported that the infected shrimps had higher percent of eosinopilic granulocytes (EG) 24.59% and pH 15.57% respectively. According to Hose and Martin (1989), large granulocytes has increased to about 41.84% in infected shrimp than normal 24.59% which is similar to present observation.

The total haemocyte count (THC) of the diseased shrimps was found to be lower than the apparently healthy shrimps. In the differential counts, the hyaline haemocytes showed a marked increase, small granulocytes were found to be decreased, and the large granulocytes showed a marginal increase. Thus the THC and DC can be taken as health indicator of shrimp.

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References

Bachère, E., E. Miahieand and J. Rodriguez : Identification of defence effectors in the haemolymph of crustaceans with particular reference to the shrimp Penaeus japonicas (Bate): prospects and application. *Fish Shellfish Immunlogy.*, **5** : 597-612 (1995).

Bauchau, A. G. : Crustaceans. In N. A. Ratcliffe and A. E Rowley (eds.): Invertebrate Blood Cells. Academic Press, New York, USA. Vol. 2, pp. 385-420 (1981).

Cerenius, L. and K. Söderhäll : The prophenoloxidase-activating system in invertebrates. *Immunology Review,* **198** : 72-82 (2004).

Cornick, J. W. and J. E. Stewart : Interaction of the pathogen *cyaffkya homari* with natural defence mechanism of *Homarus americamis. J. Fish. Res. Board Can.*, **25** : 695-709 (1968).

Fontaine, C. T., and D. V. Lightener : Observations on the process of wound repair in penacid shrimp. *J. invertebrate pathology,* **22** : 23-33 (1973).

Hennig, O., T. Itami, M. Maeda, M. Kondo, Y. Natsukari, and Y. Takahashi : Analyses of hemolymph immunoparameters in kuruma shrimp infected with penaeid rod-shaped DNA virus. *Fish Pathology,* **33** : 389-393(1998).

Hose, J. E. and G. G. Martin : Defense functions of granulocytes in the ridgeback prawn Sicyonia ingentis. *J. Invertebrate Pathol.*, **53** : 335-346 (1989).

Hose, J. E., G. G. Martin and A. S. Gerard : A decapods haemocyte classification scheme integrating morphology, cytochemistry and function *Biol. Bull.*, **178** : 33-45 (1990).

Hose, J. E., G. G. Martin and S. G. Alison : A decapod hemocyte classification scheme integrating morphology, cytochemistry and function. *The Biolological Bulletin,* **178** : 33-45 (1990b).

Hsieh, S. L., Y. H. Ruan, Li, Y. C., Hsieh, P. S. Hu, C. H. and C. M. Kuo : Immune and physiological responses in Pacific white shrimp *(Penaeus vannamei)* to *Vibrio alginolyticus. Aquaculture,* **275** : 335341 (2008).

Iwanaga, S. and B. L. Lee : Recent advances in the innate immunity of invertebrate animals. *Biochemistry and Molecular Biology,* **38** : 128-150 (2005).

Jago Jussila, Jeff Jago, Elana Tsvcntnenko, Bob Dunstan and Louis H. Evans : Total and differential haemocyte counts in western rock lobsters *Panulirus eyganus* George under post harvest stress *marine and freshwater research,* **48**(8) : 863-868 (2004).

Johanson, M. W., P. Keyser, K. Sritunyalucksana and K. Söderhäll : Crustacean hemocytes and hematopoiesis. *Aquaculture,* **191** : 4552 (2000).

Joseph, Selvina, A. J. Huxleya and A. P. Lipton : Immunomodulatory potential of marine secondary metabolites against bacterial diseases of shrimp, *Aquaculture,* **230**(8) : 241-248 (2004).

Kakoolaki, S., I. Sharifpour, M. Soltani, H. Ebrahimzadeh Mousavi and S. Mirzargar : Selected morpho-chemical features of hemocytes in farmed shrimp, *Fennero Feneropenaeus indicusin* Iran, *Iranian Journal of Fisheries Sciences.*, **9**(2) : 219-232 (2010).

Kondo, M. : Experiments of body defence mechanisms in Crustacean. Institute of Applied Aquabiology, National Fisheries University, pp.1-13 (2003).

Kondo, M., T. Itami, Y. Takahashi, R. Fujii and S. Tomonaga : Ultrastructural and cytochemical characteristics of phagocytes in Kuruma prawn. *Fish Pathology,* **33** : 421-427 (1998).

Lavine, M. D. and M. R. Strand : Insect hemocytes and their role in immunity. Insect *Biochemistry and Molecular Biology,* **32** : 1295-1309 (2002).

Lighter, D. V. : Diseases of cultured penacid shrimp. In J. P. Me vey (ED) CRC Handbook of mariculture. Vol. I Crustacean. *Aquaculture.*, CRC press Boca. Raton. FL. Pp. 289-320 (1983).

Lightner, D. V. : Shrimp virus diseases: Diagnosis, distribution and management. In Aquaculture 92. Proceedings of the Special Session on Shrimp Farming, Baton Rouge: Orlando: *The World Aquaculture Society,* pp. 238-253 (1992).

Martin, G. G. and B. L. Graves : Fine Structure and Classification of shrimp haemocytes. *J. Morphol.*, **185** : 339-348 (1985).

Moullac, Gilles Le Claude Soyez, Denis Saulnier, Dominique Ansquer, Jean Christophe Avarre, and Peva Levy : Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris. Fish & Shellfish Immunology,* Volume 8, Issue 8, November 1998, Pages 621-629 (2005).

Newman, M. C. and S. Y. Feng : Susceptibility and resistance of the rock crab, *Cancer irroratus* to natural and experimental bacterial infection. *Journal of invertebrate pathology,* **40** : 75-88 (1982).

Perazzolo, L. M., R. Gargioni, P. Ogliari and M. A. A. Barracco : Evaluation of some hematoimmunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. *Aquaculture,* **214** : 19-33 (2002).

Selvin, J., A. J. Huxley and A. P. Lipton : Pathogenicity, antibiogram and biochemical characteristics of luminescent *Vibrio harveyi,* associated with 'black shell disease' of *Penaeus monodon. Fish. Technol.,* **42** : 191-196 (2005).

Sinderman, C. J. : Diseases caused mortalities in Mariculture, Status and Predictions- proc world. *Maricult.* 80C., **2** : 69-74 (1971).

Söderhäll, K. and L. Cerenius : Crustacean immunity. *Annu Rev Fish Dis.,* **2** : 3-23 (1992).

Song Yen Ling, Chun-I Yu, Tzu-wen Lien, Chih- Chih-Cheng Huang and Mm- Nan Lin. : Haemolyph parameters of pacific white shrimp *Litopenaeus vannamei* infected with taura syndrome virus. *Fish and Shell fish Immunology,* **14** : 317-331 (2003).

Tzou, P., E. De Gregorio and B. Lemaitre : How Drosophila combats microbial infection: a model to study innate immunity and host-pathogen interactions. Current *opinion in microbiology,* **5** : 102-110 (2002).

Wood, P. J., Podlewski and T. E. Shenk : (1971) Cytochemical observation of haemolymph cells during coagulation in the Crayfish, oreonectes virilis. *J. Morphol.*, **134** : 479-488 (1971).